REMARKS

Claims 1, 3, 4, 7-9, 11-20, 22, 24, 26 and 27 remain pending in this application.

Claims 1 and 27 have been amended to recite (i) options for substituents on ring A in accordance with the disclosure at page 28, lines 5-23, for example; (2) options for Y and Ya in accordance with the disclosure at page 29, lines 11-23, for example; and (3) that R^1, R^2, R^4 and R^5 can be C_{1-6} alkyl in accordance with the disclosure at page 33, lines 12 and 30, respectively, for example. Claim 4 has been amended by deleting reference to R^3, now defined in claim 1. Claim 7 has been amended to define Yd in the manner disclosed in the specification at page 40, lines 6-28 and page 37, lines 2-20. Finally, claims 19, 20, 24 and 26 have been amended to more clearly define the disease conditions to be treated in accordance with the disclosure at page 69, lines 15-20, for example, and to delete reference to "diabetic complications" and either "prophylactic" or "preventing" disease conditions. Accordingly, no new matter has been introduced by these amendments.

Rejection: § 112, first paragraph

Claims 1, 3, 4, 7-9, 11, 12, 14-20, 22, 24, 26 and 27 have been rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the written description requirement. Although applicants disagree with the rejection, and disagree that the classification of the compound is relevant to the written restriction requirement, all claims have been amended in a sincere effort to expedite prosecution of the application. The amendments contained in this reply address each of the items identified in the rejection as raising an issue of failing to comply with the written description requirement. Accordingly, this rejection should be withdrawn.

Claims 1, 3, 4, 7-9, 11, 12, 14-20, 22, 24, 26 and 27 have been rejected as not being supported by an enabling disclosure. Although applicants disagree with this rejection, in the interest of expediting prosecution of this application, the claims have been amended to coincide with the examiner's determination of that part of the disclosure relative to the variables Y and Ya that is considered enabling. Applicants appreciate the examiner's identification of the subject matter that was regarded as enabling - very helpful in this case. Accordingly, this rejection should be withdrawn.

Claims 22, 24 and 26 have been rejected under 35 U.S.C. § 112, first paragraph, as not being supported by an enabling disclosure for the treatment of diabetes type II or obesity, and for the prevention of diabetes type 1 or 2 as well as obesity. Although applicants do not agree with this rejection, and again in the interest of expediting the prosecution of this application, these claims have been amended to delete reference to diabetes complications and to "preventing" these disease conditions.

It is respectfully submitted that the claims as amended are supported by an enabling disclosure since Boehm and Lustig, "Use of Somatostatin receptor ligands in obesity and diabetic complications," Best Practice & Research Clinical Gastroenterology, Vol. 16, No. 3, pp. 493-509 (2002) (copy attached), describes the correlation between inhibition of somatostatin (SMS) receptor binding and treatment of obesity and diabetic complications. SMS inhibits the release of growth hormone. *Boehm and Lustig* at 494. "Both obesity and diabetes mellitus are characterized by imbalances of multihormonal systems," including growth hormone and IGF-I. *Id.* SMS and SMS analogs suppress the secretion of growth hormone and reduce the level of IGF-I. *Id.* at 493. SMS and SMS analogs "inhibit insulin secretion." *Id.* at 494.

Accordingly, SMS and SMS analogs serve to lower the levels of growth hormone, IGF-I, and/or insulin.

As each of obesity and diabetes are characterized by common hormonal imbalances, one would expect that ameliorating one or more of these imbalances would provide a benefit in treating either or both of these diseases/conditions. Applicants' claimed compounds inhibit the binding of SMS to its receptor. *Specification* at 1. By inhibiting this binding interaction, endogenous SMS is prevented from decreasing the levels of growth hormone, IGF-I, and/or insulin. Thus, preventing SMS binding is a strategy for increasing the levels of these hormones. The ability to increase the levels of these hormones affords benefits to treating either or both of diabetes and/or obesity because these diseases/conditions share common causes, as discussed above. Accordingly, this rejection should be withdrawn.

Rejection: § 112, 2d paragraph

Claims 1, 3, 4, 7-9, 11, 12, 14-20, 22, 24, 26 and 27 have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite regarding the substituents for ring A, the variables R^1 and R^2 and the recitation of diabetic complications. While applicants disagree with this rejection since the terms used in these claims would have been understood by those skilled in the art and were not insolubly ambiguous, the amendments made to these claims in a sincere effort to expedite prosecution of this application are considered to avoid these rejections. Accordingly, this rejection should be withdrawn.

Prompt and favorable reconsideration of this application is respectfully requested, and timely issuance of a notice of allowance.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: March 12, 2009

By: Charles E. Van Horn
Charles E. Van Horn
Reg. No. 40,266

Attachment: Boehm and Lustig, "Use of Somatostatin Receptor Ligands in Obesity and Diabetic Complications," Best Practice & Research Clinical Gastroenterology, Vol. 16, No. 3, pp. 493-509 (2002)

Best Practice & Research Clinical Gastroenterology
Vol. 16, No. 3, pp. 493–509, 2002
doi:10.1053/bega.2002.0320, available online at http://www.idealibrary.com on IDEAL®

BEST PRACTICE & RESEARCH

10

Use of somatostatin receptor ligands in obesity and diabetic complications

Bernhard O. Boehm MD

Professor of Internal Medicine
Division of Endocrinology, Ulm University, Robert-Koch-Strasse 8, 89070, Ulm/Donau, Germany

Robert H. Lustig MD

Professor of Clinical Pediatrics
Division of Pediatric Endocrinology, University of California San Francisco, 500 Parnassus Avenue, San Francisco, CA 94143-0136, USA

Somatostatin (SMS) is a potent inhibitory molecule. It inhibits both exocrine and endocrine secretory functions of the pancreas, suppresses growth hormone secretion and reduces the level of insulin-like growth factor-1. Long-acting somatostatin analogues were currently investigated for potential clinical benefits in two settings: (a) control of hyperinsulinaemia in obesity and (b) control of an excess of pro-angiogenic factors in diabetes-associated retinal complications. In two randomized, controlled trials the long-acting somatostatin analogue octreotide retarded progression of the microvascular complications in pre-proliferative and advanced stages of diabetic retinopathy. Inhibition of the early phase of insulin secretion by use of octreotide in patients with hypothalamic obesity resulted in weight loss and improved quality of life. Efficacy of octreotide correlated to residual β-cell activity prior to the treatment. Obesity and diabetes mellitus are the most common chronic metabolic disorders in the world. The use of somatostatin analogues addressing the various hormonal imbalances of these disorders may provide a novel concept for their pharmacological treatment.

Key words: diabetic retinopathy; hyperinsulinaemia; hypothalamic obesity; IGF-system; obesity; somatostatin analogues; somatostatin receptors.

Obesity and diabetes mellitus are the most common chronic metabolic disorders in the world.[1] Both diseases can be found in almost all populations. Obesity is a chronic metabolic disorder characterized by an excess of body fat. The classification of obesity is based on the International Obesity Task force recommendation which was made in 1997: pre-obese subjects (body mass index [BMI] 25–29.9), class I obesity (BMI 30–34.9), class II obesity (BMI 35–39.9) and class III obesity as a BMI equal to or greater than 40. The classification defines a strong association of obesity-related co-morbidity. Obesity is associated with increased mortality and morbidity due to hypertension, type 2 diabetes, coronary artery disease, stroke, gallbladder diseases, musculoskeletal problems, including osteoarthritis, sleep apnoea, and other respiratory problems. Various hormonal complications have been described, including menstrual irregularities, hirsutism associated with hyperandrogenaemia, and complications of pregnancy. Also,

1521–6918/02/$ - see front matter

prevalence of some cancer types (colon, prostate, breast, endometrium) is associated with an excess of body fat.

There is a clear-cut association of an altered glucose metabolism with obesity, duration of obesity and weight gain. Changes in insulin secretion resulting in hyperinsulinaemia and insulin resistance of peripheral tissues provide important clues in the development of diabetes mellitus. However, diabetes mellitus itself is a heterogeneous group of disorders characterized by a chronic elevated blood glucose level and the propensity to develop specific micro- and macrovascular complications. Typical microvascular complications are diabetic nephropathy and diabetic retinopathy. Macrovascular complications lead to an increased risk for myocardial infarction, stroke gallblader disease, osteoarthritis, sleep apnoea and limb amputations. Type 2 diabetes is the most common form of diabetes in adults, although the incidence of type 2 diabetes in childhood has risen dramatically by more than 300% in the past 5 years.[2]

Both obesity and diabetes mellitus are characterized by imbalances of multihormonal systems. Obesity is a phenotype of many diseases; however, hyperinsulinaemia, is a hallmark of obesity. By manipulating this hormonal dysbalance, obesity may become a potentially treatable disease. Both the naturally occurring molecule somatostatin and SMS analogues inhibit insulin secretion.[3,4] Therefore these molecules may be attractive drugs in the treatment of hyperinsulinaemia.

As in obesity, diabetes mellitus is also characterized by multihormonal imbalances, one of which refers to growth hormone (GH) and the IGF-I system.[5–7] Because growth hormone and IGF-I system components act as permissive factors of pro-angiogenic signals, their modulation and/or inhibition by use of the inhibitory substance peptide somatostatin, or by SMS analogues, is also an attractive target for the treatment of vascular complications of diabetes mellitus with neoangiogenesis.

SOMATOSTATIN (SMS)

The peptide somatostatin (SMS) was defined in the 1960s as a molecule that inhibits the release of growth hormone. The molecule was found almost accidentally during studies of the distribution of growth-hormone-releasing factor in the hypothalamus of rats.[8,9]

SMS is synthesized as a precursor molecule. After enzymatic cleavage, two active peptides, somatostatin-28 and somatostatin-14, are generated. These molecules are ligands for a specific set of somatostatin-binding receptors (sstrs).[10,11]

Somatostatin can be found throughout the brain and spinal cord and also in the intestine. Like somatostatin, its receptors have been found to be located ubiquitously throughout the body.[12] However, the various subtypes of receptor are differentially expressed at the target sites of SMS action. Five subtypes of receptor have been described thus far, each of which is encoded by one of five independent genes located on different chromosomes. The biological effects are mediated by these five membrane-bound specific receptors, called sstr 1–5 (Tables 1 and 2). All receptors are G protein-coupled receptors with seven transmembrane spanning domains linked to adenylate cyclase.[13,14]

The physiological actions of somatostatin are primarily inhibitory[13,14] (Tables 1, 2). SMS affects, for example, calcium and potassium ion channels, leads to tyrosine phosphatase activation and may therefore modulate transmission of neuroendocrine cells, modulate their secretion, and may also affect cell proliferation at several organ systems. Effects have recently been found on the retina and vascular endothelial

Table 1. Actions of somatostatin.

Organ	Action
Pituitary	GH ↓, TSH ↓
Endocrine pancreas	Insulin ↓, Glucagon ↓ Pancreatic Polypetide ↓
Exocrine pancreas	Bicarbonate secretion ↓, Enzyme secretion ↓
Thyroid	T4 ↓, T3 ↓, Calcitonin secretion ↓
Gastrointestinal tract	Mesenteric blood flow ↓
	Mucosal cell proliferation ↓
	Gastrin ↓, Secretin ↓, CCK ↓, VIP ↓, GIP ↓
	Motilin ↓, Enteroglucagon ↓, Neurotensin ↓
	Gastric acid ↓, Pepsin ↓, Intrinsic factor ↓
	Gastric emptying ↓
	Gallbladder contraction ↓
	Small intestine segmentation ↓
	Colonic fluid secretion ↓
	Bile secretion ↓

cells.[6,15] SMS acts as a classical hormone, a neurohormone, as a neurotransmitter and exerts autocrine and paracrine functions.

SMS analogues

The naturally occurring somatostatin has a very short half-life of only 1.5–3 minutes; this pharmacological peculiarity requires a continuous intravenous route for the application of SMS. A post-infusion hypersecretion of growth hormone is also found. To address these limitations synthetic analogues of the molecule have been developed.[4] Each of the synthetic analogues incorporates four amino acids in a specific configuration. The various somatostatin receptor agonists bind to each of the sstr subtypes with varied affinities (Table 3). Only a very recently described analogue

Table 2. Somatostatin receptor subtype expression.

Subtype	1	2	3	4	5
Chromosomal location	14	17	22	20	16
Distribution					
1. Brain	+	+	+	+	+
2. Eye	−	+	−	−	−
3. Kidneys	+	−	−	−	−
4. Exocrine Pancreas	+	−	+	−	−
Endocrine Pancreas	−	+	−	−	+
5. Pituitary	−	−	−	−	+
6. Heart	−	−	−	−	+
G protein coupling	+	+	+	+	+
K^+/Ca^{2+} channels	−	+	−	−	−
Adenyl cyclase coupling	+	+	+	+	+

(SOM230) was found to bind almost like the naturally occurring SMS peptides to all sstrs with equal affinity to exert equal potency.[16] Non-peptidergic receptor analogues may provide a non-parenteral usage of SMS analogues in clinical medicine.[17]

DIABETES MELLITUS

Progressive damage to the eyes, kidneys, nerves and large vessels represents the major threat to health and life of diabetic patients[1,18] Therefore, prevention of complications and disability due to the chronic hyperglycaemic state should be undertaken in order to alleviate the burden of diabetes-specific complications.[1]

Diabetic retinopathy

Retinopathy is the most frequent and specific chronic microvascular complication of diabetes mellitus. It represents a major threat to eyesight in the Western countries, and is the leading cause of blindness among people of working age.[19,20] Although strict glucose control can delay the onset of diabetic retinopathy and ameliorate the severity of the disease, both in type 1 and type 2 diabetics, epidemiological data show that the incidence of diabetic retinopathy remains strikingly high.[19–21]

Diabetic retinopathy (DR) can be classified into three stages: non-proliferative retinopathy, which does not impair vision, proliferative retinopathy, which impairs the vision, and ongoing proliferations of the vasculature which may lead to vitreo-retinal surgical approaches to preserve some vision. Loss of vision due to diabetic retinopathy involves several mechanisms. Central vision may be impaired by either macular oedema, or ischaemic maculopathy. New pathological blood vessels, which appear at the proliferative phase of the disease, lead to visual impairment by vitreous haemorrhage. The development of proliferative vitreoretinopathy accompanied by contraction of the fibrous tissue can distort the retina, thus leading to tractional retinal detachment.

After 20 years, diabetic retinopathy can be detected in almost all patients with type 1 diabetes mellitus (80–95%) and in a majority of type 2 diabetic patients (50–80%).

Glucose hypothesis of diabetic retinopathy and its prevention

Two large randomized prospective clinical trials showed the important role of glucose levels in the development of diabetic complications ('glucose hypothesis'). Intensive conventional insulin therapy (ICT) in type 1 diabetic patients reduced or prevented the development of retinopathy by 27% as compared with conventional therapy (primary prevention) in the Diabetes Control and Complications Trial (DCCT).[22] Glycosylated haemoglobin 1c (HBA1c) levels in the intensified treated group were about 7.0% versus 9.0% in the conventionally treated group; almost half of the patients in the intensified treated group reached an HbA1c level of 6.1% or less at least once during the study period. Thus, intensified insulin regimen significantly diminished the progression of diabetic retinopathy (secondary prevention). Most importantly, early treatment with ICT was most effective.

In the United Kingdom Prospective Diabetes Study (UKPDS) trial an intensified treatment (insulin treatment and/or use of various oral agents) of newly diagnosed type 2 diabetic patients lowered HbA1c to a median level of 7.0% and decreased the probability of microvascular complications by 25%.[23]

Table 3. In vitro binding to human recombinant somatostatin receptors.

Subtype	1	2	3	4	5
Peptide					
SRIF-14	2.26	0.23	1.16	1.76	1.41
SRIF-28	2.38	0.29	1.26	2.93	0.40
Lanreotide	2414	0.75	97.8	1826	5.21
Octreotide	876	0.57	26.7	5030	6.77

Ki (nM) are given; a low number reflects high-affinity binding. Data indicate that both SMS analogues bind with high affinity to sstr 2 and sstr 5 receptors.

From both trials it has become clear that lowering glucose levels per se justifies the widespread use of the so-called intensified treatment regimens in diabetics. Lowering of blood glucose levels was found to be effective in both type 1 and type 2 diabetic patients in lowering the likelihood of the presentation and the progression of microvascular complications. However, no evidence for a glucose threshold could be found.[24–26] If glucose lowering fails, photocoagulation is an important treatment modality. However, after photocoagulation visual acuity may decrease and constriction of peripheral visual field occurs.[19,20] It is still a matter of debate whether so-called early photocoagulation in patients with mild-to-severe non-proliferative or early proliferative diabetic retinopathy is justified.

Growth hormone and the IGF-1 system

Diabetes mellitus is characterized by multihormonal imbalances which include the growth hormone (GH) and IGF-1 system. GH has been implicated as an important mediator of diabetic retinopathy. Diabetic patients with a life-long growth hormone deficiency rarely develop retinopathy. In addition, pituitary ablation in patients with retinopathy has been shown to lead to an improvement of this microvascular complication.[7,19,27]

Alterations in secretion of growth hormone, altered response to the hypothalamic suppressor somatostatin, excess of growth hormone-releasing hormone (GHRH), or an altered responsiveness to insulin-like growth factor I (IGF-I), which also contributes to the negative feedback loop of the GH secretion, are all implicated in the observed changes in growth hormone levels.[4]

The development of DR is a multifactorial process with a disturbed milieu of growth factors and inhibitory molecules which can be produced locally and which can also result from the vasculature due to the breakage of the blood–retina barrier. It was shown recently that the vasculature of the normal retina is maintained by the action of a natural anti-angiogenic factor derived from the pigment epithelium. This factor, which has been named pigment epithelium derived factor (PEDF) controls the vasculature and maintains its quiescent state even when the strong pro-angiogenic factor VEGF is increased.[28,29] A low level of PEDF was found to strongly predict the development of either pre-proliferative or proliferative retinopathy. A reciprocal fall in inhibitory PEDF was needed before pro-angiogenic factors could act as stimulatory molecules to the vasculature. It appears that PEDF acts as a brake on VEGF and other stimulatory factors and that it is only when this brake fails that pathological retinopathy can develop.[28,30,31]

In addition, insulin-like growth factor components play an important role as permissive factors for VEGF action, and the inhibition of this molecule was shown to control neo-angiogenesis in an animal model of retinopathy.[6]

Treatment of diabetic retinopathy with somatostatin and somatostatin analogues

Somatostatin or somatostatin analogues can suppress the secretion induced by growth hormone releasing hormone (GHRH).[3,4] In the 1960s and 1970s – when somatostatin was not yet available – inhibition of GH secretion by hypophysectomy was found to be a clinically effective treatment of DR.[32]

Table 4 summarizes the treatment protocols published thus far which have used somatostatin analogues in the context of diabetic retinopathy (summarized in ref. 33; Table 4). Various dosages of the somatostatin analogues (minimal dosage per day 150 μg, maximal dosage per day 500 μg of SMS 201–995; 1500 μg/day of BMI23014) have been used in patients with proliferative retinopathy[33] and cystoid macular oedema.[33,34] Some studies reported effects on the suppression of growth hormone levels, stabilization of neovascularizations, resorption of haemorrhages and the reduction in the number of microaneurysms. In a case report, effective treatment of a macular oedema was also reported.

Recently, two controlled trials have been reported. Grant and co-workers have studied patients with severe non-proliferative diabetic retinopathy or early non-high-risk proliferative retinopathy.[35] At this stage of diabetic retinopathy the likelihood for the need of panretinal photocoagulation is high. The SMS analogue octreotide was titrated in 11 patients to the maximally tolerated dose for a 15-month period. Doses of 200 μg/day up to 5000 μg/day of octreotide were used. Only one of 22 eyes of octreotide-treated patients required panretinal photocoagulation, whereas nine of 24 eyes in the control group had to be laser treated. The incidence of ocular disease progression was only 27% in patients treated with octreotide compared with 42% in patients with conventional management. This study provided evidence that octreotide treatment retarded progression of advanced retinopathy and delayed the time for laser photocoagulation. Boehm et al reported the use of octreotide in a cohort of diabetic patients with a very advanced stage of proliferative diabetic retinopathy, i.e. presence of active proliferations after full scatter laser treatment.[36] A dose of 300 μg/day of octreotide was used in nine patients; nine patients with standard diabetes management served as controls. The observation period was the longest ever reported in a trial using a somatostatin analogue for the treatment of diabetic complications. After 3 years of treatment the incidence of vitreous haemorrhages was significantly lower in the octreotide-treated patients. Also, visual acuity was preserved and significantly better over time in the octreotide-treated group. Only in the group of octreotide-treated patients did a regression of proliferations, as defined by stereoscopic photography and by the use of fluorescein angiography, occur.

All trials have carefully monitored the safety of SMS use[30–32] (Table 4). In the Ulm trial no severe hypoglycaemic events, as defined by help needed from third parties or requirement of hospital admissions, occurred during an observational period of almost 3 years. Two patients complained of abdominal discomfort and increased bowel movements, which could be alleviated by the use of an oral pancreatic enzyme supplementation. No gall bladder stones or sludges were noted on the routine ultrasound examinations of the abdomen.[36]

Ongoing clinical trials are currently evaluating the possible effect of a sustained release form of octreotide in patients with severe non-proliferative and early proliferative

Table 4. Trials using somatostatin and somatostatin analogues for the treatment of diabetic retinopathy.

Author and citation	Number of patients	Treatment	Duration	Effects	Side-effects
Uncontrolled trials					
Mallet et al (1992)	4 (type I)	SMS 201–995 continuous s.c. infusion 44 μg/day	6–20 (mean 15) months	Two patients: neovascularizaation stopped Two patients: neovascularizaation regressed	Insulin doses decreased, digestive disturbances, gallbladder stones (1)
Lee et al (1998)	Case report (type I)	SMS 201–995 3 × 50 μg s.c. per day	3 months	Complete resorption of haemorrhages Reduction in numbers of microaneurysms and areas of extracapillary dye leakage	None
Shumak et al (1990)	6 (type I)	SMS 201–995 3 × 100 μg per day	8 weeks	Improvement of visual acuity, improvement of retinopathy in two patients	None
Kuijpers et al (1998)	Case report	SMS 201–995 s.c. 3 × 100 μg per day	12 weeks	Macular oedema dried, visual acuity improved, but: rebound effect	
Controlled trials					
Hyer et al (1989)	9 (type I) (6 controls)	(A) SMS 201–995 3 × 50 μg s.c. per day (B) Continuous s.c. infusion 500 μg/day	(A) 20 weeks (B) 3 days to 16 weeks	(A) No GH suppression, visual acuity minus 2 lines in 3/4 patients (B) Complete/partial suppression of GH levels, no change to visual acuity (5 patients), improvement (1 patient). No prevention of retinal haemorrhages and further laser treatment	Abdominal discomfort, trend to hypoglycaemia

Table 4 continued over page

Table 4. Continued

Author and citation	Number of patients	Treatment	Duration	Effects	Side-effects
Kirkegaard et al (1990)	7 (11) (9 controls)	SMS 201–995 continuous s.c. infusion 400 μg/day	12 months	No effects, no difference between the two groups	Decline in insulin requirements, mild transient hypothyroidism
McCombe et al (1991)	11 (6 controls)	BMI123014 continuous s.c. infusion 1500 μg/day	3 months	Two patients: clinical and angiographic improvement; six patients: no effect	
Grant et al (2000)	11 (12 controls)	SMS 201–995 s.c. 200–5000 μg/day	15 months	Progression to high-risk proliferative retinopathy was significantly reduced; progression was seen in 27% of SMS treated patients, in 42% of controls	Decline in insulin requirements, replacement therapy with thyroxine
Boehm et al (2001)	9 (9 controls)	SMS 201–995 s.c. 300 μg/day	36 months	Episodes of vitreous haemorrhages and need for vitreoretinal surgery were reduced significantly by use of SMS	Up to 40% decline in insulin requirements in type I patients

Mallet et al (1992) *Diabete et Metabolisme* **18**: 438; Lee et al (1998) *Diabetes Care* **11**: 441; Shumak et al (1990) *Clinical and Investigative Medicine* **13**: 287; Kunjipers et al (1998) *New England Journal of Medicine* **338**: 624; Hyer et al (1989) *Acta Endocrinologica* **120**: 187; Kirkegaard et al (1990) *Acta Endocrinologica* **122**: 766; McCombe et al (1991) *Eye* **5**: 569; Grant et al (2000) *Diabetes Care* **23**: 504; Boehm et al (2001) *Hormone and Metabolic Research* **33**: 300.

diabetic retinopathy. The effect on macular oedema is also studied because case reports demonstrated that somatostatin analogues inhibit vascular effusions.[15,34]

Other effects of SMS on eye diseases

The expression pattern of both somatostatin and its receptor subtype 2 on other cellular elements of the human eye makes it highly likely that SMS exerts direct regulatory functions.[15] The observed positive effects in cystic maculopathy and the positive case reports in patients with macular oedema make it likely that SMS controls fluid evasion or resorption.[31] Most probably the pigment epithelium cell, which can produce pro- and anti-angiogenic molecules, is responsible for a favourable exchange of fluids.[27,28] SMS was also used in age-related macular degeneration. It was found to act in the so-called presumed ocular histoplasmosis syndrome, thus extending the modulatory effects of SMS in other ocular neovascularization diseases.[30,36]

The future role of pharmacotherapy of diabetic retinopathy

The long-acting analogue octreotide (SMS 201–995), as well as other SMS analogues, provides an elegant pharmacological principle to modify the high-risk form of proliferative diabetic retinopathy.[30] The potential role of these substances may be due to suppression of pro-angiogenic molecules, a direct inhibition of pro-angiogeneic signalling at the cell level, an anti-fibrotic action, thus reducing fibrovascular formations, and at least a partial correction of the systemic growth hormone and IGF-I dysregulation.[5–7,15,30,31,37–39] Data from the Ulm trial and from other pilot investigations have provided evidence that octreotide can very effectively suppress new bleedings and stop visual loss in patients who have failed conventional photocoagulation therapy.[30,35,37] In this cohort octreotide was found to be a safe treatment modality. It remains to be clarified whether the progression from pre-proliferative to proliferative retinopathy can also be stopped by the use of the long-acting somatostatin analogues.[15,30,40,41]

OBESITY

The incidence of obesity worldwide is climbing at an alarming rate.[42] The fallacy of obesity is that it can be attributable to either increased caloric intake or decreased caloric expenditure only. In fact, the aetiologies and pathogeneses of obesity are myriad[43], as are the potential therapies[44], but a rational approach to obesity is dependent on the development of a useful nosology with consistent and accurate diagnostic criteria.

One clear feature shared by all obesity subtypes is an excess of adipose tissue. Excess blood-borne energy substrates (glucose, fatty acids) which are not oxidized immediately by other tissues are stored in adipose via the lipogenic effects of the hormone insulin. The amplitude and duration of pancreatic insulin secretion and the activity of the insulin molecule at the adipose insulin receptor both play important roles in the genesis of lipogenesis and weight gain. This is supported by the fact that hyperinsulinaemia is a component of most animal models of obesity (eg. *ob*, *db*, *fa*, *Ay*).[45] Insulin is the primary hormonal mediator of adipogenesis in humans.[46] Within the adipocyte, insulin regulates: (a) Glut4 expression; (b) acetyl-CoA carboxylase; (c) fatty acid synthase; and (d) lipoprotein lipase.[47] The overwhelming majority of obese humans exhibit hyperinsulinism and insulin resistance as hallmarks of their obesity.[48,49]

Hyperinsulinism, insulin resistance, and obesity are inexorably linked though reciprocal control mechanisms. Hyperinsulinaemia may cause insulin resistance through changes in glucose transport[50,51] or through insulin's down-regulation of its own receptor[52,53], and can cause lipogenesis and increased weight gain directly, as is noted in infants of diabetic mothers.[54,55] Conversely, insulin resistance may cause compensatory hyperinsulinaemia through poorly understood reflex mechanisms[56], and can cause lipogenesis through up-regulation of the Glut4 transporter and the enzymes acetyl-CoA carboxylase and fatty acid synthase.[47,57] Lastly, the degree of obesity exhibits a direct correlation with the degree of insulin resistance.[58,59] Although the debate continues on which comes first – the insulin secretion, the insulin resistance, or the obesity[60,61] – these concepts clearly suggest that insulin is a potential target for obesity pharmacotherapy.[62] Acute glucose-stimulated insulin hypersecretion in insulin-sensitive adults has been shown to predict future weight gain.[63] Similarly, in children, an augmented early post-prandial insulin response precedes the development of obesity.[64] Such studies lend credence to the hypothesis that insulin hypersecretion is a risk factor for the development of obesity in humans.

Neural regulation of β-cell function

The regulation of β-cell function is extremely complex; in this process neural, endocrine, and paracrine factors all interact to control insulin secretion. The ventromedial hypothalamus (VMH) coordinates energy balance through both sympathetic and parasympathetic inputs to the β-cell. Damage to the VMH in rats leads to hyperphagia, hyperinsulinaemia and obesity.[65–68] There is ample evidence that abnormally increased vagal tone with resultant insulin hypersecretion mediate this phenomenon.[69–78] The vagus may also promote insulin hypersecretion through stimulation of intestinal L-cells to produce the incretin glucagon-like peptide-1 (GLP-1), which reaches the β-cell haematogenously and binds to specific receptors to augment the insulin response to glucose.[79,80]

Somatostatinergic regulation of β-cell function

Rodent β-cells possess sstr-5 receptors, as determined by immunocytochemical[81], molecular biological[82] and combinatorial chemical[17] methods; however, in humans there is still some debate as to whether the subtype sstr 2 predominates. In both species, the sstr is coupled to the voltage-gated calcium channel, and SMS exerts an inhibitory action on channel opening.[83] In the presence of SMS or analogue, the magnitude of the calcium influx is attenuated, which limits the amplitude of the early insulin response to glucose. Acutely, SMS suppression of insulin secretion may cause hyperglycaemia[84], but this is rapidly normalized within a few days, while the suppression of the early insulin response to glucose limits the compartmentalization of energy substrate into adipose.

The use of somatostatin analogues in obesity

SMS and its analogues have ubiquitous physiological actions which can be exploited through selective binding to different sstr subtypes.[13–16] Nowhere is this better exemplified than in the use of the SMS analogue octreotide for the treatment of a subtype of obesity due to vagal dysregulation of pancreatic β-cell function and insulin excess.

Octreotide therapy of hypothalamic obesity

Experimental bilateral electrolytic lesions or de-afferentation of the VMH in rats leads to hyperphagia and intractable weight gain, termed 'hypothalamic obesity'.[71,85,86] Similarly in humans, hypothalamic damage from a tumour, surgery or radiation often results in unrelenting weight gain, which is not amenable to caloric restriction or exercise.[66,87–90] Hypothalamic obesity is probably due to VMH neuronal damage with an alteration in leptin feedback.[67] Indeed, caloric restriction of either VMH-lesioned animals or patients with hypothalamic obesity does not attenuate the weight gain[91], suggesting that energy expenditure is also affected. Patients with hypothalamic obesity manifest an increase in vagal firing rate which promotes insulin hypersecretion.[72,73] The excess insulin causes shunting of blood-borne energy substrate to adipose.[90] Furthermore, leptin, insulin and ghrelin resistance due to hypothalamic damage would prevent any afferent feedback effects on caloric intake, leading to continued weight gain.[67] This would result in an obesity syndrome with high leptin, low fasting insulin, and high and early peak insulin on OGTT.

We analysed the insulin excursion to oral glucose tolerance testing (OGTT) in eight children with intractable obesity secondary to tumour therapy.[92] Our subjects exhibited only slightly elevated fasting insulin concentrations. However, they demonstrated accentuated early insulin responses to OGTT, which were elevated in comparison to their degree of obesity. Peak insulin levels were reached by 60 minutes, and were 281 $\pm$ 47 μU/ml, as compared to 8–150 μU/ml in normal adolescents. The fall to baseline was rapid, suggesting that these patients did not exhibit defective insulin clearance, as is usually seen with obesity due to insulin resistance.[59]

The early phase of insulin secretion can be pharmacologically inhibited by limiting the opening of the voltage-gated calcium channel using the SMS analogue octreotide.[4] In an open-labelled pilot trial[92], eight patients with hypothalamic obesity received subcutaneously administered octreotide for 6 months, at a dose of 5 μg/kg/day tid, escalating in monthly 5 μg/kg/day increments to a maximum of 15 μg/kg/day tid. Insulin responses to glucose were normalized over 6 months. Of the eight patients, three lost substantial weight, two lost moderate amounts of weight, and three stabilized their weight. The degree of weight loss correlated both with changes in insulin response and changes in leptin levels. The weight loss also appeared to correlate with decreases in appetite as caloric intake in this cohort decreased by approximately 700 kcal/day. The lack of leptin negative feedback by the damaged VMH was not critical as these patients responded with weight loss, decreased caloric intake and decreased leptin levels. In fact, an unexpected but welcome side-effect of the treatment was the resumption of normal physical activity by four patients, including vigorous exercise.

A double-blind, placebo-controlled 6-month trial of octreotide in 18 subjects with hypothalamic obesity has now been completed.[93] Prior to treatment, annualized weight gain of these patients was 15.9 $\pm$ 2.9 kg/year. Although the weight loss in this trial was not as pronounced, octreotide was effective in stabilizing weight ($+1.6 \pm 0.6$ kg) and BMI (-0.1 ± 0.1 kg/m^2), as compared to placebo (change in weight $+9.2 \pm 1.5$ kg, BMI $+2.3 \pm 0.5$ kg/m^2). Insulin secretion was clearly suppressed by octreotide therapy. Lastly, a measurement of quality of life demonstrated marked improvements in physical activity as compared with those treated with placebo. These data suggest that insulin hypersecretion is responsible not only for weight gain in these patients, but for their lack of physical activity, which can be improved by normalizing their insulin response. Anecdotally, patients noted normal hunger at the time of meals but noted the lack of drive to continue to eat past their

first serving, or for snacking between meals. They also noted more interest in their schoolwork and in their social contacts. Treatment of those initially assigned to placebo resulted in stabilization of weight and improved quality of life as well.

Octreotide therapy of adult obesity due to primary insulin hypersecretion

We hoped to identify a subpopulation of obese adults who also demonstrated insulin hypersecretion[63] but without obvious cranial pathology. Forty-four severely obese (BMI > 35 kg/m^2) but otherwise healthy adults were treated in an open-label fashion with octreotide-LAR, 40 mg intramuscularly every 28 days for a 6-month period, without changes in lifestyle, diet or exercise.[94] Of the 44 subjects, eight exhibited substantial weight loss (mean 13 kg). The eight responders exhibited significantly different baseline insulin dynamics to OGTT compared to the other 36 subjects, with a rapid insulin rise peaking at 60 minutes, and an equally rapid fall. Octreotide suppressed this insulin rise, without glucose intolerance. Again, the change in leptin correlated with the change in BMI, suggesting reduction in adipose stores. The degree of pre-treatment β-cell activity was an a priori predictor of response to octreotide: the higher the pre-treatment β-cell activity the more weight was lost. Furthermore, vagal modulation correlated directly with β-cell activity in these obese patients.[95]

Of course, this does not preclude other possible mechanisms of octreotide action to reduce weight and BMI. Modulation of other GI hormones, such as glucagon-like peptide-1[79,80]; slowing of gastric emptying and GI motility, with nutrient malabsorption[96]; direct effects on appetite[97,98], or direct effects on the adipocyte[99,100] have all been reported with SMS or its analogues. However, these other mechanisms seem less likely, as GI symptoms and appetite suppression were uniformly distributed throughout all response strata (data not shown), and only those subjects who exhibited weight loss demonstrated insulin suppression, as exhibited by decreases in C-peptide and insulin excursions, and decreases in IAUC.[101] Furthermore, if another mechanism, other than insulin suppression, was responsible for the weight loss, subjects receiving octreotide for acromegaly or other disorders would be expected to lose weight and fat mass; indeed, long-term octreotide usage has minimal effects on these parameters.[102] Lastly, SMS has been shown to reduce food intake in animals only

Table 5. Applications of somatostatin analogues.

Established applications
Pituitary adenomas: acromegaly, TSHoma
Metastatic islet-cell tumours
Metastatic neuroendocrine tumours
Somatostatin-receptor scintigraphy
Chemotherapy-induced diarrhoea
Acute oesophageal varicea bleeding
Probable applications
Pancreatic and enteric fistulas
Secretory diarrhoea
AIDS-related diarrhoea
Possible applications, clinical applications under investigation
Diabetic microvascular complications: retinopathy, nephropathy
Obesity due to insulin hypersecretion
Graves ophthalmopathy
Radiotherapy of somatostatin-receptor-positive diseases

if their vagus nerve is left intact.[98] This suggests that the decline in caloric intake with SMS or its analogues is mediated through suppression of β-cell depolarization.

SUMMARY

Obesity and diabetes mellitus are the most common chronic metabolic disorders in the world. A massive increase of insulin secretion is typical of obesity, whereas an increase of growth hormone secretion and changes in components of the IGF-I system are paradigmatic of the dysglycaemic syndrome. Because the actions of somatostatin and somatostatin analogues are primarily inhibitory, these molecules may provide novel treatment modalities. However, before the widespread use of these inhibitory molecules can occur, our understanding of the pathophysiology of obesity and diabetes mellitus has to be changed. For example, diabetes mellitus not only requires the substitution of insulin for a balanced glucose level but also a direct modification of the imbalance of the IGF-I system. From a historical standpoint these are new implications of concepts almost 30 years old.[103,104] The relative merits of SMS treatment of obesity and diabetic complications remain to be assessed in large multicentre investigations. Nevertheless, currently available SMS analogues have already allowed the modulation of the expression of obesity and the expression of diabetic retinopathy at various stages of disease and, as such, provide a novel concept in endocrinological practice (Table 5).

Practice points

- obesity and diabetes mellitus are characterized by imbalances of multihormonal systems, including insulin, and growth hormone and IGF-I system components
- long-term somatostatin analogue treatment is safe and can retard progression of retinopathy and preserve vision
- long-term somatostatin analogue treatment can retard weight gain and suppress hyperinsulinaemia in obesity

Research agenda

- clinical research will need to demonstrate the efficacy of somatostatin analogue treatment for prevention and retardation of diabetic retinopathy
- clinical research will need to demonstrate the efficacy of somatostatin analogues in the treatment of obesity, including the demonstration of safety in long-term trials

REFERENCES

1. Report of a WHO Study Group. Prevention of Diabetes mellitus. WHO Technical Report Series, Geneva, 1994.
2. Pinhas-Hamiel O, Dolan L, Daniels S et al. Increased incidence of non-insulin-dependent diabetes mellitus among adolescents. *Journal of Pediatrics* 1996; **128**: 608–615.
3. Gerich JE, Lorenzi M, Schneider V et al. Effects of somatostatin on plasma glucose and glucagon levels in human diabetes mellitus. Pathophysiologic and therapeutic implications. *New England Journal of Medicine* 1974; **291**: 544–547.

* 4. Lamberts SWJ, van Der Lely AJ, De Herder WW et al. Drug therapy: octreotide. *New England Journal of Medicine* 1996; **334:** 246–254.

5. Feldmann B, Jehle PM, Mohan S et al. Diabetic retinopathy is associated with decreased serum levels of free IGF-I and changes of IGF-binding proteins. *Growth Hormone & IGF Research* 2000; **10:** 53–60.

* 6. Smith LE, Kopchick JJ, Chen W et al. Essential role of growth hormone in ischemia-induced retinal neovascularization. *Science* 1997; **276:** 1706–1709.

7. Poulsen SE. The Houssay phenomenon in man: recovery from retinopathy in a case of diabetes with Simmond's disease. *Diabetes* 1953; **2:** 7–12.

8. Krulich L, Dhariwal APS & McCann SM. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. *Endocrinology* 1968; **83:** 783.

9. Brazeau P, Vale W, Burgus R et al. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 1972; **179:** 77.

* 10. Yamada Y, Post SR, Wang K et al. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. *Proceedings of the National Academy of Sciences of the USA* 1992; **89:** 251–255.

11. Yamada Y, Reisine T, Law SF et al. Somatostatin receptors, an expanding gene family: cloning and functional characterization on human SSTR3, a protein coupled to adenly cyclase. *Molecular Endocrinology* 1992; **6:** 2136–2142.

12. Raulf F, Perez J, Hoyer D & Bruns C. Differential expression of five somatostatin receptor subtypes, SSTR1–5, in the CNS and peripheral tissue. *Digestion* 1994; **55 (s):** 46–53.

13. Patel YC. Molecular pharmacology of somatostatin receptors subtypes. *Journal of Endocrinological Investigation* 1997; **20:** 348–367.

* 14. Hoyer D, Lübbert H & Bruns C. Molecular pharmacology of somatostatin receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology* 1994; **350:** 441–453.

15. van Hagen PM, Baarsma GS, Mooy CM et al. Somatostatin and somatostatin receptors in retinal diseases. *European Journal of Endocrinology* 2000; **143:** S43–S51.

16. Bruns C, Lewis I, Briner U & Weckbecker G. SOM230: a new somatostatin peptidomimetic with unique binding and endocrine profile. Endocrine Society Meeting, Denver CO 2001 (abstract).

17. Rohrer SP, Birzin ET, Mosley RT et al. Rapid identification of subtype-selective agonists of the somatostatin receptor through combinatorial chemistry. *Science* 1998; **282:** 737–740.

18. Report of a WHO Consultation. *Definition, diagnosis and classification of diabetes mellitus and its complications*. World Health Organization, Geneva, 1999.

19. Klein R. The epidemiology of eye disease. In Pickup JC & Williams G (eds) *Textbook of Diabetes*, 2nd edn, pp 44.1–44.9. Oxford: Blackwell Sciences, 1997.

20. Kohner EM. Diabetic retinopathy. *British Medical Journal* 1993; **307:** 1195–1199.

21. Klein R, Klein BEK, Moss SE & Cruickshanks KJ. The Wisconsin Epidemiologic study of diabetic retinopathy. XIV. Ten-year incidence and progression of diabetic retinopathy. *Archives of Ophthalmology* 1994; **112:** 1217–1228.

22. The Diabetes Control and Complications Trial Research Group, The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *New England Journal of Medicine* 2000; **342:** 381–389.

23. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; **352:** 837–853.

24. DCCT Research Group. The absence of a glycemic threshold for the development of long-term complications: the perspective of the Diabetes Control and Complications Trial. *Diabetes* 1996; **45:** 1289–1298.

25. UKPDS 33. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. *Lancet* 1998; **352:** 837–853.

26. Stratton IM, Adler AI, Neil HA et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *British Medical Journal* 2000; **321:** 405–412.

27. Poulsen SE. Diabetes and anterior pituitary insufficiency: final course and postmortem study of a diabetic patient with Sheehan's syndrome. *Diabetes* 1966; **15:** 73–77.

28. Dawson DW, Volpert OV, Gillis P et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* 1999; **285:** 245–248.

* 29. Stellmach V, Crawford SE, Zhou W & Bouck N. Prevention of ischemia induced retinopathy by the natural ocular anti-angiogenic agent pigment epithelium-derived factor. *Proceedings of the National Academy of Sciences of the USA* 2001; **98:** 2593–2597.

30. Aiello LP, Avery RL, Arrigg PG et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *New England Journal of Medicine* 1994; **331:** 1480–1487.
31. Aiello LP, Pierce EA, Foley ED et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proceedings of the National Academy Sciences of the USA* 1995; **92:** 10 457–10 461.
32. Kohner EM, Hamilton AM, Joplin GF & Fraser TR. Florid diabetic retinopathy and its response to treatment by photocoagulation or pituitary ablation. *Diabetes* 1976; **25:** 104–110.
33. Boehm BO. Treatment of diabetic retinopathy with long-acting somatostatin analogues. In Lamberts SWJ (ed.) *Octreotide: The next decade*, pp 241–257. Bristol: BioScientifica Ltd, 1999.
34. Kujipers RWAM, Baarsma GS & van Hagen PM. Treatment of cystoid macular edema with octreotide. *New England Journal of Medicine* 1998; **338:** 624–626.
35. Grant MB, Mames RN, Fitzgerald C et al. The efficacy of octreotide in the therapy of severe nonproliferative and early proliferative retinopathy. A randomized controlled study. *Diabetes Care* 2000; **23:** 504–509.
36. Shternfeld IS, Chambers RB, O'Dorisio TM et al. The effect of octreotide on ocular proliferative disease: a pilot study. *Investigation Ophthalmology and Visual Science* 1999; **40:** S70.
* 37. Boehm BO, Lang GK, Jehle PM et al. Octreotide reduces risk for vitreous hemorrhages and loss of visual acuity in patients with high risk proliferative diabetic retinopathy. *Hormone Metabolic Research* 2001; **33:** 300–306.
38. Suzuma I, Hata Y, Clermont A et al. Cyclic stretch and hypertension induce retinal expression of vascular endothelial growth factor and vascular endothelial growth factor-2: potential mechanisms for exacerbation of diabetic retinopathy by hypertension. *Diabetes* 2001; **50:** 444–454.
39. Adamis AP, Miller JW, Bdernal M-T et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *American Journal of Ophthalmology* 1994; **118:** 445–450.
40. Lawnicka H, Stepien H, Wyczolkowaska J et al. Effect of somatostatin and octreotide on proliferation and vascular endothelial growth factor secretion from murine endothelial cell line (HECCa10) culture. *Biochemical and Biophysical Research Communications* 2000; **268:** 567–571.
41. Demir T, Celiker UO, Kukner A et al. Effect of octreotide on experimental corneal neovascularization. *Acta Ophthalmologica Scandinavica* 1999; **77:** 386–390.
42. Mokdad AH, Serdula MK, Dietz WH et al. The spread of the obesity epidemic in the United States. 1991–1998. *Journal of the American Medical Association* 1999; **282:** 1519–1522.
* 43. Lustig RH. The neuroendocrinology of obesity. *Endocrinology and Metabolism Clinics of North America* 2001; **30:** 765–785.
44. Bray GA & Gallagher TF. Manifestations of hypothalamic obesity in man: a comprehensive investigation of eight patients and a review of the literature. *Medicine* 1975; **54:** 301–333.
45. York DA. Lessons from animal models of obesity. *Endocrinology and Metabolism Clinics of North America* 1996; **25:** 781–800.
46. Marin P, Russeffé-Scrive A, Smith J & Bjorntorp P. Glucose uptake in human adipose tissue. *Metabolism* 1988; **36:** 1154–1164.
47. Ramsay TG. Fat cells. *Endocrinology and Metabolism Clinics of North America* 1996; **25:** 847–870.
48. Lillioja S, Nyomba B, Saad M et al. Exaggerated early insulin release and insulin resistance in a diabetes-prone population: a metabolic comparison of Pima Indians and caucasians. *Journal of Clinical Endocrinology and Metabolism* 1991; **73:** 866–876.
49. Haffner SM, D'Agostino R, Saad MF et al. Increased insulin resistance and insulin secretion in non-diabetic African-Americans and Hispanics compared with non-Hispanic whites: the insulin resistance atherosclerosis study. *Diabetes* 1996; **45:** 742–748.
50. Postic C, Burcelin R, Rencurel F et al. Evidence for a transient inhibitory effect of insulin of GLUT2 expression in the liver: studies in vivo and in vitro. *Biochemical Journal* 1993; **293:** 119–124.
51. Thorens B, Flier JS, Lodish HF & Kahn BB. Differential regulation of two glucose transporters in rat liver by fasting and refeeding and by diabetes and insulin treatment. *Diabetes* 1990; **39:** 712–719.
52. Kolterman OG, Reaven GM & Olefsky JM. Relationship between in vivo insulin resistance and decreased insulin receptors in obese man. *Journal of Clinical Endocrinology and Metabolism* 1979; **48:** 487–494.
53. Knutson VP. Proteolytic processing of the insulin receptor beta subunit is associated with insulin-induced receptor down-regulation. *Journal of Biological Chemistry* 1991; **266:** 15 656–15 662.
54. Silverman BL, Landsberg L & Metzger BE. Fetal hyperinsulinism in offspring of diabetic mothers: association with the subsequent development of childhood obesity. *Annals of the New York Academy of Sciences* 1993; **699:** 36–45.
55. Krew MA, Kehl RJ, Thomas A & Catalano PM. Relation of amniotic fluid C-peptide levels to neonatal body composition. *Obstetrics and Gynecology* 1994; **84:** 96–100.

* 56. Reaven GM. Pathophysiology of insulin resistance in human disease. *Physiological Reviews* 1995; **75:** 473–486.
57. Assimacopoulos-Jeannet F, Brichard S, Rencurel F et al. In vivo effects of hyperinsulinemia on lipogenic enzymes and glucose transporter expression in rat liver and adipose tissues. *Metabolism* 1995; **44:** 228–233.
58. Barakat HA, Mooney N, O'Brien K et al. Coronary heart disease risk factors in morbidly obese women with normal glucose tolerance. *Diabetes Care* 1993; **16:** 144–149.
59. Bonadonna RC, Groop L & Kraemer N. Obesity and insulin resistance in humans: a dose response study. *Metabolism* 1990; **39:** 452–459.
60. Ravussin E & Swinburn BA. Insulin resistance is a result, not a cause of obesity: Socratic debate: the pro side. In Angel A et al (ed.) *Progress in Obesity Research*, pp 173–178. London: Libbey, 1996.
61. Sims EAH. Insulin resistance is a result, not a cause of obesity: Socratic debate: the con side. In Angel A et al (ed.) *Progress in Obesity Research*, pp 587–592. London: Libbey, 1996.
62. Campfield LA, Smith FJ, Mackie G et al. Insulin normalization as an approach to the pharmacological treatment of obesity. *Obesity Research* 1995; **3:** 591S–603S.
63. Sigal RJ, El-Hashimy M, Martin BC et al. Acute post-challenge hyperinsulinemia predicts weight gain. *Diabetes* 1997; **46:** 1025–1029.
64. LeStunff C & Bougneres P. Early changes in postprandial insulin secretion, not in insulin sensitivity, characterize juvenile obesity. *Diabetes* 1994; **43:** 696–702.
65. Jeanrenaud B. An hypothesis on the aetiology of obesity: dysfunction of the central nervous system as a primary cause. *Diabetologia* 1985; **28:** 502–513.
66. Sorva R. Children with craniopharyngioma: early growth failure and rapid post-operative weight gain. *Acta Pediatrica Scandinavica* 1988; **77:** 587–592.
67. Satoh N, Ogawa Y, Katsura G et al. Pathophysiological significance of the *obese* gene product, leptin in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. *Endocrinology* 1997; **138:** 947–954.
68. Bray GA & York DA. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiological Reviews* 1979; **59:** 719–809.
69. Gilon P & Henquin JC. Mechanisms and physiological significance of the cholinergic control of pancreatic β-cell function. *Endocrine Reviews* 2001; **22:** 565–604.
70. Ionescu E, Rohner-Jeanrenaud F, Berthoud HR & Jeanrenaud B. Increases in plasma insulin levels in response to electrical stimulation of the dorsal motor nucleus of the vagus nerve. *Endocrinology* 1983; **112:** 904–910.
71. Berthoud HR & Jeanrenaud B. Acute hyperinsulinemia and its reversal by vagotomy following lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology* 1979; **105:** 146–151.
72. Tokunaga K, Fukushima M, Kemnitz JW & Bray GA. Effect of vagotomy on serum insulin in rats with paraventricular or ventromedial hypothalamic lesions. *Endocrinology* 1986; **119:** 1708–1711.
73. Inoue S & Bray GA. The effect of subdiaphragmatic vagotomy in rats with ventromedial hypothalamic lesions. *Endocrinology* 1997; **100:** 108–114.
74. Miura Y, Gilon P & Henquin JC. Muscarinic stimulation increases Na^+ entry in pancreatic β-cells by a mechanism other than the emptying of intracellular Ca^{2+} pools. *Biochemical and Biophysical Research Communications* 1996; **224:** 67–73.
75. Zawalich WS, Zawalich KC & Rasmussen H. Cholinergic agonists prime the β-cell to glucose stimulation. *Endocrinology* 1989; **125:** 2400–2406.
76. Rohner-Jeanrenaud F & Jeanrenaud B. Involvement of the cholinergic system in insulin and glucagon oversecretion of genetic preobesity. *Endocrinology* 1985; **116:** 830–834.
77. Tian YM, Urquidi V & Ashcroft SJH. Protein kinase C in β-cells: expression of multiple isoforms and involvement in cholinergic stimulation of insulin secretion. *Molecular and Cellular Endocrinology* 1996; **119:** 185–193.
78. Blondel O, Bell GI, Moody M et al. Creation of an inositol 1,4,5-triphosphate-sensitive Ca^{2+} store in secretory granules of insulin-producing cells. *Journal of Biological Chemistry* 1994; **269:** 27 167–27 170.
79. Kiefer TJ & Habener JF. The glucagon-like peptides. *Endocrine Reviews* 1999; **20:** 876–913.
80. Rocca AS & Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* 1999; **140:** 1687–1694.
81. Mitra SW, Mezey E, Hunyady B et al. Colocalization of somatostatin receptor sst5 and insulin in rat pancreatic β-cells. *Endocrinology* 1999; **140:** 3790–3796.
82. Gordon DF, Woodmansee WW, Lewis SR et al. Cloning of the mouse somatostatin receptor subtype 5 gene: promoter structure and function. *Endocrinology* 1999; **140:** 5598–5608.
83. Hsu WH, Xiang HD, Rajan AS et al. Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the beta-cell. *Journal of Biological Chemistry* 1991; **266:** 837–843.

84. Giustina A, Bussi AR, Pizzocolo G et al. Acute effects of octreotide, a long-acting somatostatin analog, on the insulinemic and glycemic responses to a mixed meal in patients with essential obesity: a dose-response study. *Diabetes, Nutrition, and Metabolism* 1994; **7:** 35–41.
85. Rohner-Jeanrenaud F & Jeanrenaud B. Consequences of ventromedial hypothalamic lesions upon insulin and glucagon secretion by subsequently isolated perfused pancreases in the rat. *Journal of Clinical Investigation* 1980; **65:** 902–910.
86. Bray GA, Inoue S & Nishizawa Y. Hypothalamic obesity. *Diabetologia* 1981; **20:** 366–377.
87. Bray GA. Syndromes of hypothalamic obesity in man. *Pediatric Annals* 1984; **13:** 525–536.
88. Lustig RH. Hypothalamic obesity: the sixth cranial endocrinopathy. *Endocrinologist* 2002 (in press).
89. Stahnke N, Grubel G, Lagenstein I & Willig RP. Long-term follow-up of children with craniopharyngioma. *European Journal of Pediatrics* 1984; **142:** 179–185.
90. Pinto G, Bussieres L, Recasens C et al. Hormonal factors influencing weight and growth pattern in craniopharyngioma. *Hormone Research* 2000; **53:** 163–169.
91. Bray GA & Nishizawa Y. Ventromedial hypothalamus modulates fat mobilization during fasting. *Nature* 1978; **274:** 900–902.
* 92. Lustig RH, Rose SR, Burghen GA et al. Hypothalamic obesity in children caused by cranial insult: altered glucose and insulin dynamics, and reversal by a somatostatin agonist. *Journal of Pediatrics* 1999; **135:** 162–168.
93. Lustig RH, Hinds PS, Smith KR et al. A double-blind, placebo-controlled trial of octreotide in pediatric hypothalamic obesity, 6th Lawson Wickins Pediatric Society/European Pediatric Endocrine Society meeting, 2001; (abstract).
94. Velasquez-Mieyer PA, Buffington CK, Arheart KL et al. Primary insulin hypersecretion: a specific and remediable subtype of obesity, 11th International Congress of Endocrinology, 2000; p 1054 (abstract).
95. Cashion AK, Velasquez-Mieyer, PA, Arheart KL et al. Correlations between autonomic function, β-cell activity, insulin sensitivity, and glucose tolerance in obesity, 11th International Congress of Endocrinology, 2000; p 1056.
96. Simsolo RB, Ezzat S, Ong JM et al. Effects of acromegaly treatment and growth hormone on adipose tissue lipoprotein lipase. *Journal of Clinical Endocrinology and Metabolism* 1995; **80:** 3233–3238.
97. Lotter EC, Krinsky R, McKay JM et al. Somatostatin decreases food intake of rats and baboons. *Journal of Comparative and Physiological Psychology* 1981; **95:** 278–287.
98. Levine AS & Morley JE. Peripherally administered somatostatin reduces feeding by a vagal mediated mechanism. *Pharmacology, Biochemistry, and Behavior* 1982; **16:** 897–902.
99. Simón MA, Romero B & Calle C. Characterization of somatostatin binding sites in isolated rat adipocytes. *Regulatory Peptides* 1988; **23:** 261–270.
100. Campbell RM & Scanes CG. Inhibition of growth hormone-stimulated lipolysis by somatostatin, insulin, and insulin-like growth factors (somatomedins) in vitro. *Proceedings of the Society for Experimental Biology and Medicine* 1988; **189:** 362–366.
101. Jiminez J, Zuniga-Guajardo S, Zinman B & Angel A. Effects of weight loss in massive obesity on insulin and C-peptide dynamics: sequential changes in insulin production, clearance, and sensitivity. *Journal of Clinical Endocrinology and Metabolism* 1987; **64:** 661–668.
102. Hansen TB, Gram J, Bjerre P et al. Body composition in active acromegaly during treatment with octreotide: a double-blind, placebo-controlled cross-over study. *Clinical Endocrinology* 1994; **41:** 323–329.
*103. Luft R & Guillemin R. Growth hormone and diabetes in man. Old concepts – new implications. *Diabetes* 1974; **23:** 783–787.
104. Gerich JE, Lorenzi M, Schneider V et al. Inhibition of pancreatic glucagon responses to arginine by somatostatin in normal man and in insulin-dependent diabetics. *Diabetes* 1974; **23:** 876–880.